Dykema Docket No. 065379-0053

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Joerg ROSENBERG *et al.*		Examiner:	Jennifer Y. CHO
Serial No.:	10/539,505	Group Art Unit No.:	1621
Filing Date:	December 16, 2003	Confirmation No.:	4705

For:

FORMULATION COMPRISING FENOFIBRIC ACID, A PHYSIOLOGICALLY ACCEPTABLE SALT OR DERIVATIVE THEREOF

DECLARATION UNDER 37 C.F.R. SECTION 1.132 of GUENTER BLAICH

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Guenter Blaich, hereby declare as follows:

1. I received my Diploma and Ph.D in Biochemistry from University Tübingen in 1981 and 1984, respectively. I have been employed by Abbott GmbH & Co. KG ("Abbott GmbH"), Ludwigshafen, Germany, the assignee of the above-identified application, since 2001. Specifically, from September 2001 through April 2003, I served as the Head of Toxicology. From May 2003 – August 2007, I served as Scientific Director, Global Preclinical Safety, first for Abbott Laboratories[1] ("Abbott"; from May 2003 – January 2006) and then for Abbott GmbH (from February 2006 – August 2007). Since August 2007, I have served as Director, Preclinical Safety and Scientific Director, Global Preclinical Safety for Abbott GmbH.

In approximately June or July 2003, I became responsible for Abbott's fenofibric acid development program.

My curriculum vitae is attached herewith as Exhibit A.

2. Although I am not an inventor, I have read the above-identified application and Office Action mailed on September 17, 2007.

3. I have also read the article by Gurrieri, J. et al., *Drug Res.*, 26(5) (1976) (hereinafter "Gurrieri"), a copy of which is attached herewith as Exhibit B. Gurrieri describes the hypolipidemic drug, isopropyl-[4'-(p-chlorobenzoyl)-2-phenoxyl]-propionate (LF 178 (fenofibrate)) and studies involving this drug and its major circulating metabolite, phenoxy-isobutryic acid (fenofibric acid), in rats. Specifically LF 178 and the sodium salt of phenoxy-isobutryic acid (LF 153) was administered to rats in doses ranging from 50-300 mg/kg. As shown in Figure 2, LF 153 had significant side effects at doses exceeding 200 mg/kg. Moreover, Gurrieri provides no toxicokinetic data for LF 178 or LF 153. As far as I am aware and to the best of my knowledge, no one skilled in the art disputed the results of this article.

4. In December 1997, Abbott and Fournier Pharma[2] ("Fournier") signed an agreement to develop, register, market, use and sell fenofibrate and metabolites thereof (including fenofibric acid).

5. Although I was not present, I became aware that in April 2001, during a Fournier fenofibric acid development meeting, the Gurrieri article was discussed. According to notes sent to me from this meeting, the following comment was made, "The dosage administered in the 1976 article far exceeded the amount normally used to reduce lipids in rats (50 mg/kg). Nevertheless, a further safety and pharmacology study will be needed to identify the safe dosages of FFA[3] in rats."

6. In August and October 2001, toxicology and pharmacokinetic studies were performed by Fournier using micronized fenofibrate, fenofibric acid and salts of fenofibric acid.

In one study, the gastric ulcerogenic effect and pharmacokinetics of micronized fenofibrate ("FEN") and fenofibric acid ("FA") were evaluated. Specifically, male and female rats were orally administered a single dose of 100 mg/kg, 300 mg/kg or 1000 mg/kg of micronized fenofibrate or fenofibric acid. The rats were placed on a water-only fast the day prior to the study. The pharmacokinetic data from the study is shown below in Table A.

Table A

Test compound	Dose (mg/kg)	Cmax (μg/mL) Males/Females	AUC (μg•h/mL) Males/Females
FEN	100	85.7/243	1757/5752
	300	195/391	4875/10132
	1000	310/417	7528/12382
FA	100	396/454	5414/11135
	300	579/688	12382/22353
	1000	903/1075	29223/42646

[1] Abbott Laboratories is the parent company of Abbott GmbH.
[2] Fournier Pharma is based in France. In 2005, Solvay S.A. purchased Fournier Pharma.
[3] FFA refers to fenofibric acid.

Administration of 1000 mg/kg of fenofibric acid to the rats was found to be lethal. With respect to toxicology, statistically significant ulcerogenic activity was found in rats administered 1000 mg/kg of fenofibric acid. The damage was located in the corpus of the stomach. At the lower doses of 100 and 300 mg/kg, no statistically significant ulcerogenic activity was observed.

In a second study, the gastric ulcerogenic effect and pharmacokinetics of fenofibric acid ("FA"), the lysine salt of fenofibric acid ("FA-Lysine") and the arginine salt of fenofibric acid ("FA-Arginine") were evaluated. Specifically, male and female rats were orally administered a single dose of 300 mg/kg or 1000 mg/kg of fenofibric acid, the lysine salt of fenofibric acid or the arginine salt of fenofibric acid following a 4-day observation period. The pharmacokinetic data from the study is shown below in Table B.

Table B

Test compound	Dose (mg/kg)	Cmax (μg/mL) Males/Females	AUC (μg•h/mL) Males/Females
FA (Reference)	1000	767/951	23896/46314
FA-Lysine	300	517/761	12327/26652
	1000	869/1262	25957/11351#
FA-Arginine	300	487/695	10601/23382
	1000	865/1198	16032/11544#

- Lethal dose with limited PK

Administration of 1000 mg/kg of fenofibric acid and the lysine and arginine salts of fenofibric acid to the rats was found to be lethal. With respect to toxicology, statistically significant ulcerogenic effects were found in rats administered 300 mg/kg and 1000 mg/kg of the lysine and arginine salts of fenofibric acid. Statistically significant gastric ulcerations were also found in rats administered 1000 mg/kg of fenofibric acid.

In a third study, a two week gavage study was performed with female rats. In this study, a tube was inserted into the mouth of the rats who were orally administered 100 mg/kg, 300 mg/kg or 500 mg/kg of micronized fenofibrate or fenofibric acid for 14 consecutive days. The pharmacokinetic data from the study is shown below in Table C.

Table C

Test compound	Dose (mg/kg/day)	Cmax (μg/mL)	AUC (μg•h/mL)
FEN	100	NA	5442
	300	NA	10632
	500	NA	13915
FA	100	NA	9386
	300	NA	14312
	500	NA	16678

NA: Not available.

Statistically gastric ulcerations were found in rats administered 300 mg/kg and 500 mg/kg of fenofibric acid.

7. During another Fournier fenofibric acid development meeting in December 2001, the above studies were discussed. Although I was not present during this meeting, according to notes sent to me from the meeting, the following comment was made, "Studies shown the potential of ulcerogenicity with fenofibric acid at higher doses (1000 mg/kg) which may be due both to a local effect, but also to a higher systemic exposition."

8. In January 2002, another Fournier fenofibric acid development meeting was held. Although I was not present during this meeting, according to the notes sent to me from the meeting, a comment was made that a formal dose ranging study was required to understand the gastrointestinal side effects associated with fenofibric acid. My understanding is that at the time of this meeting it was believed that there was a problem with fenofibric acid in terms of side effects and that formulation work was necessary.

9. In January 2002, Abbott retained Hugh E. Black & Associates, Inc. ("Hugh Black") to review the data that Fournier had generated regarding fenofibric acid to advise as to a possible developmental pathway for fenofibric acid.

10. In September 2002, a meeting was held at Abbott to review the recommendations of Hugh Black. Hugh Black recommended that Abbott conduct a 90 day toxicity study with fenofibric acid.

11. As mentioned previously herein, in approximately June or July 2003, I became responsible for Abbott's fenofibric acid development program. At that time, my understanding was that the gastrointestinal side effects associated with fenofibric acid were still an issue, and that Abbott needed to conduct studies to examine and understand the nature of these side effects.

From November 2003 through March 2005, five (5) studies were performed by Abbott to examine the gastrointestinal side effects associated with fenofibric acid and salts of fenofibric acid. In these studies, the gastric ulcerogenic effect and pharmacokinetics of micronized fenofibrate ("FEN"), fenofibric acid ("FA") and the calcium and choline salts of fenofibric acid were evaluated. The first study was a 5-week palatability study. In this study, the rats were administered Rodent Chow, 100 mg/kg/day or 300 mg/kg/day of micronized fenofibrate. The second study was a 5-week palatability study. In this study, the rats were administered Rodent Chow, 10 mg/kg/day, 30 mg/kg/day, 75 mg/kg/day or 150 mg/kg/day of fenofibric acid. The third study was a 2-week gavage study. In this study, the rats were administered Rodent Chow, 30 mg/kg/day, 100 mg/kg/day or 300 mg/kg/day of the calcium salt of fenofibric acid ("FA-Calcium"). The fourth study was a 2-week gavage study. In this study, the rats were administered Rodent Chow, 30 mg/kg/day, 100 mg/kg/day or 300 mg/kg/day of the

choline salt of fenofibric acid ("FA-Choline"). The fifth study was a 3-month gavage study. In this study, the rats were administered Rodent Chow, 10 mg/kg/day, 30 mg/kg/day or 100 mg/kg/day of the FA-Choline. The pharmacokinetic data for the highest doses of fibrofibrate, fenofibric acid or the calcium and choline salts of fenofibric acid is provided below in Table D.

Table D

Test compound	Study Type	Dose (mg/kg/day)	Cmax (μg/mL) Males/Females	AUC (μg•h/mL) Males/Females
FEN	5-week palatability	300	677/842	15274/19333
FA	5-week palatability	150	611/787	14423/20237
FA-Calcium	2-week gavage	300	682/650	14171/13452
FA-Choline	2-week gavage	300	702/747	12148/15305
FA-Choline	3-Month gavage	100	609/679	12021/12194

In each of the above studies, no gastric ulcerations were detected at any dose.

12. The Gurrieri article and the Fournier study involving the lysine and arginine salts of fenofibric acid describe gastric ulcerations in rats administered fenofibric acid and salts of fenofibric acid at doses of 300 mg/kg/day and higher. The first and second studies conducted by Abbott (See, Paragraph 11) were designed to match the exposure levels (namely, AUC) seen in the two week gavage study performed by Fournier the results of which are shown above in Table C (See, Paragraph 6). In these two studies, the exposure levels exceeded the anticipated AUCs (based on the Fournier study) but nonetheless, the rats did not show any evidence of gastric ulcerations. The 2-week gavage studies performed by Abbott using the calcium and choline salts of fenofibric acid produced plasma exposure levels between 12148 and 15305 μg•h/mL, but in none of these studies were any gastric ulcerations in the rats observed. Finally, a 3 month rat study was conducted using lower dosages of the choline salt of fenofibric acid[4]. In this study, plasma exposure levels between 12021 and 12194 μg•h/mL resulted, but no gastric ulcerations in the rats were observed. Thus, despite plasma exposure levels up to 20237 μg•h/mL and Cmax levels up to 787 μg/mL, no gastric ulcerations were observed in the Abbott studies involving fenofibric acid or the calcium and choline salt of fenofibric acid. The reason for the different outcome between the Abbott studies and the studies conducted by Fournier is unknown and was very surprising.

13. This declaration summarizes my views regarding the above topics. It is not intended to be comprehensive. As such, I reserve the right to modify or supplement the statements made herein.

[4] The lower dosages of the choline salt of fenofibric acid were used in this study because of other types of toxicity, namely, skeletal muscle and myofiber degeneration and decreased body weight gain.

14. I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.

03-Oct-2008
Date

Guenter Blaich

APPENDIX A

CURRICULUM VITAE: Guenter Blaich

Office	Abbott GmbH & Co. KG Knollstrasse 67061 Ludwigshafen/Germany Phone: +49 (0)621 589 2702 Fax: +49 (0)621 589 1185 Email: guenter.blaich@abbott.com

Nationality	German
Linguistic proficiency	English fluent French good knowledge
Education	
1973	University Entrance Qualification (Abitur)
1973 - 1975	Military Service
1975 - 1980	1975 - 1980, Biochemistry, University Tübingen 1981: Diploma in Biochemistry (Diplom-Biochemiker) "In vitro-protein binding of paracetamol metabolites in the mouse liver" (lab group Prof. Dr. A. Wendel, Institut of Physiological Chemistry, University Tübingen)
1981 - 1984	February 1984: Ph.D., biochemistry (Dr. rer. nat.); Ph.D. dissertation "Effect of phosphate and ruthenium red on calcium in rat liver mitochondria" (lab group Prof. Dr. E. Pfaff, Institut of Toxicology (Director: Prof. Dr. H. Remmer), University Tübingen) Scholarship "Studienstiftung des Deutschen Volkes"
July 84 - June 85	Post doc, Institut of Working and Social Welfare, University Tübingen (Director: Prof. Dr. F. W. Schmahl); research area "Endotoxin shock in rabbits"
1985 - 1989	Post doc, Institut of Toxicology (Director: Prof. Dr. D. Henschler), University Würzburg; Project "Metabolic activation of estrogens and its relevance for the carcinogenic effect of these compounds" (lab group Prof. Dr. M. Metzler)
Pharmaceutical industry	Jan 89, Knoll AG, Department of Drug Toxicology

1989 – 1996	• Head of Rodent Toxicology group, study director and monitor of studies at CROs (rodents and monkeys) • Head of the new Cellular Toxicology lab, establishment of in vitro methods from all relevant animal species for liver enzyme induction (phase I and II) and oxidative stress • Project team member of toxicology for various projects
1997 - Mar 2000	• Study monitor for studies at CROs in the field of Genetic and Reproductive Toxicology • Head of Cellular and Molecular Toxicology group, expansion of methodology (thyroid homöostasis) • Project team member of toxicology for various projects • Research project team member of toxicology for various research projects
Jan - Mar 2000	• Preclinical project leader, reporting to Global Head of Pharmaceutical Center, BASF Pharma
From Apr 2000	• Head of Experimental Toxicology/Pathology, reporting to Global Head of Toxicology, BASF Pharma • Head of Preclinical Safety Subteam • Research project team member of toxicology for various research projects
From Sep 2001 – April 2003	• Head of Toxicology, Abbott GmbH & Co. KG, Ludwigshafen; reporting to Global Head Toxicology, Abbott Laboratories, AP, IL
May 2003 – January 2006	• Scientific Director, Global Preclinical Safety; Abbott Laboratories, Abbott Park, IL
February 2006 – August 2007	• Scientific Director, Global Preclinical Safety; Abbott GmbH & Co. KG, Ludwigshafen/Germany
August 2007 - present	• Director, Preclinical Safety and Scientific Director, Global Preclinical Safety; Abbott GmbH & Co. KG, Ludwigshafen/Germany
Further educations	• 16 Mar 1993: Certified toxicologist of the German Society of Pharmacology and Toxicology (DGPT) • September 1999: Certified toxicologist/EUROTOX

Societies memberships

- Deutsche Gesellschaft für experimentelle und klinische Pharmakologie und Toxikologie (DGPT)
- Gesellschaft für Umwelt-Mutagenese (GUM)
- Society of Toxicology (SOT)
- Drug Information Association (DIA)

Publications See listing appendix I

Posters/lectures See listing appendix II

APPENDIX I:

Publications

1 Krell H, Täfler M, Blaich G and Pfaff E (1984)
On the state of calcium ions in isolated rat liver mitochondria. I. Ion fluxes and volume changes upon calcium uptake under various conditions.
Hoppe-Seyler's Z Physiol Chem 365, 59 - 71

2 Blaich G, Krell H, Täfler M and Pfaff E (1984)
On the state of calcium ions in isolated rat liver mitochondria. II. Effects of phosphate and pH on calcium-induced release.
Hoppe-Seyler's Z Physiol Chem 365, 73 - 82

3 Blaich G, Krell H and Pfaff E (1984)
On the state of calcium ions in isolated rat liver mitochondria. III. Diversity of ruthenium red action on different calcium pools.
Hoppe-Seyler's Z Physiol Chem 365, 763 - 771

4 Blaich G, Krell H and Pfaff E (1985)
On the state of calcium ions in isolated rat liver mitochondria. IV. On the prevention of phosphate-induced mitochondrial destruction by ruthenium red-insensitive calcium release.
Biol Chem Hoppe-Seyler 366, 515 - 519

5 Blaich G, Krell H and Pfaff E (1986)
On the state of calcium ions in isolated rat liver mitochondria. V. Development of a rapidly dischargeable pool of mitochondrial calcium during calcium-induced transition.
Biol Chem Hoppe-Seyler 367, 1153 - 1158

6 Bauer B, Blaich G, Metzler B and Schmahl F W (1986)
Reference values for free amino acids and other biochemical constituents in serum of male rabbits.
J Clin Chem Clin Biochem 24, 861 - 862

7 Blaich G, Pfaff E and Metzler M (1987)
Metabolism of diethylstilbestrol in hamster hepatocytes.

Biochem Pharmacol 36, 3135 - 3140

8 Blaich G, Göttlicher M, Cikryt P and Metzler M (1987)
Effect of 7,8-benzoflavone pretreatment on diethylstilbestrol metabolism, drug-metabolizing enzymes and the aromatic hydrocarbon (Ah) receptor in male hamster liver
Eur J Drug Meta Pharmacokin 12, 259 - 262

9 Krell H, Blaich G, Fromm H and Pfaff E (1988)
Role of a rapidly dischargeable pool of calcium in the transition of isolated mitochondria. In "Cellular calcium and phosphate transport in health and disease"
(Alan R Liss Inc), 171 - 176

10 Blaich G and Metzler M (1988)
The effects of pretreatment with 7,8-benzoflavone on drug-metabolizing enzymes and diethylstilbestrol metabolism in male hamster liver microsomal preparations.
Xenobiotica 18, 199 - 206

11 Blaich G and Metzler M (1988)
Effect of pretreatment with 7,8-benzoflavone and diethylstilbestrol on the hepatic metabolism of diethylstibestrol in the male Syrian golden hamster in vivo.
Biochem Pharmacol 37, 3565 - 3570

12 Blaich G, Göttlicher M, Cikryt P and Metzler M (1988)
Induction of P450 isoenzyme activities in Syrian golden hamster liver compared to rat liver as probed by the rate of 7-alkoxyresorufin-O-dealkylation.
Chem-biol Interaction 67, 129 - 138

13 Blaich G and Metzler M (1988)
Effect of pretreatment of male Syrian golden hamsters with 7,8-benzoflavone and with diethylstilbestrol on P450 isoenzyme activities and on microsomal diethylstilbestrol metabolism
J Steroid Biochem 31, 971 - 978

14 Metzler M, Tritscher A and Blaich G (1989)
Steroid-related liver tumors: Experimental induction and modulation. In: Liver cell carcinoma (P Bannasch, D Keppler and G Weber, Eds), Falk-Symposium 51, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 217 - 226

15 Blaich G and Metzler M (1989)
Possible role of metabolic activation of 7,8-benzoflavone in the formation of hamster hepatic tumors following treatment with 7,8-benzoflavone and diethylstibestrol. In: "Cytochrome P-450: Biochemistry and biophysics (ed. by I Schuster), Taylor and Francis, pp 771 - 774

16 Blaich G and Metzler M (1989)
Metabolic activation of diethylstibestrol and 7,8-benzoflavone in hamster hepatocytes. In: "Biological monitoring of exposure and the response at the subcellular level of toxic substances". Arch Toxicol, Suppl 13, 203 – 205

17 Blaich G, Göttlicher M, Cikryt P and Metzler M (1990)
Effects of various inducers on diethylstibestrol metabolism, drug-metabolizing enzyme activities and the aromatic hydrocarbon (ah) receptor in male Syrian golden hamster liver.
J Steroid Biochem 35, 201 - 204

18 Blaich G, Raabe H and Metzler M (1990)
Modification of 7,8-benzoflavone metabolism in hamster liver and kidney microsomes by hepatic inducing treatments.

Carcinogenesis 11, 95 – 101

19 Metzler M, Blaich G and Tritscher AM (1990)
Role of metabolic activation in the carcinogenicity of estrogens: Studies in an animal liver tumor model.
Environmental Health Perspectives 88, 117 - 121

20 Degen G, Blaich G and Metzler M (1990)
Multiple pathways for the oxidative metabolism of estrogens in Syrian hamster and rabbitt kidney.
J Biochem Toxicol 5, 91 – 98

21 Blaich G, Janssen B, Roth G and Salfeld J (2007)
Overview: Differentiating Issues in the Development of Macromolecules compared with small Molecules.
In: Handbook of Pharmaceutical Biotechnology, edited by Shayne Cox Gad, 2007 John Wiley & Sons, Inc.
Chapter 1.3, 89 - 121

APPENDIX II:

Abstracts (poster presentations/lectures)

1 Wendel A, Reiter R, Hricko HM, Feuerstein S and Blaich G (1982)
Modulation of drug metabolism by dietary selenium in the mouse
12th Linderstrom-Lang Conference on Selenium, Iceland, June 26 - 28

2 Blaich G and Wendel A (1983)
Hepatotoxicity and covalent binding of paracetamol in Selenium-deficient mice.
Erwin-Riesch-Symposium, Tübingen, April 20 - 22

3 Krell H, Blaich G and Pfaff E (1984)
On the nature of ruthenium red-prevented destructive calcium release from isolated rat liver mitochondria.
Intracellular Ca regulation, Satellite Symposium to the 9th International Congress of Pharmacology, Ulm/Neu-Ulm, August 6 - 8

4 Blaich G, Krell H and Pfaff E (1984)
Ruthenium red-insensitive calcium efflux from rat liver mitochondria results from two distinct processses.
Intracellular Ca regulation, Satellite Symposium to the 9th International Congress of Pharmacology, Ulm/Neu-Ulm, August 6 - 8

5 Blaich G and Metzler M (1987)
Pretreatment with 7,8-benzoflavone affects drug-metabolizing enzymes and diethylstilbestrol metabolism in male hamster liver microsomes.
28. Frühjahrstagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Mainz, March 10 - 13

6 Blaich G, Göttlicher M, Cikryt P and Metzler M (1987)
Effect of 7,8-benzoflavone pretreatment on diethylstibestrol metabolism, drug-metabolizing enzymes and the aromatic hydrocarbon (Ah) receptor in the male hamster.
ISSX 2nd European Meeting on foreign compound metabolism, Frankfurt/Main, March 29 - April 3

7 Blaich G and Metzler M (1987)
Metabolism of diethylstilbestrol in hamster hepatocytes.
IX meeting of the European Association for Cancer Research, Helsinki/Finland, May 31- June 3

8 Blaich G and Cikryt P (1987)

Effects of various inducers on hepatic estrogen metabolism, drug metabolizing enzymes and the aromatic hydrocarbon receptor.
2nd Turku Symposium on environmental estrogens, Turku/Finland, June 9

9 Blaich G and Metzler M (1987)
Modulation of enzyme activities and diethylstilbestrol metabolism in the hamster liver.
2nd Biennal Gordon Research Conference on Hormonal Carcinogenesis, New Hampton/New Hamshire, USA, August 2 - 7

10 Blaich G (1998)
Modulation of enzyme activities and diethylstilbestrol metabolism in the hamster liver.
29. Frühjahrstagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Mainz, March 8 - 11

11 Blaich G and Metzler M (1988)
Role of metabolic activation of diethylstilbestrol and 7,8-benzoflavone in the formation of hamster hepatic tumors.
6th International Congress on biochemistry and biophysics of cytochrome P-450, Vienna, July 3 - 8

12 Metzler M, Tritscher AM and Blaich G (1988)
Steroid-related liver tumours: experimental induction and modulation.
Falk-Symposium on liver cell carcinoma, Freiburg, June 5 - 8

13 Blaich G and Metzler M (1988)
Metabolic activation of diethylstilbestrol and 7,8-benzoflavone in hamster hepatoctyes.
The 29th Congress of the European Society of Toxicology and the 3rd Congress of the Federation of the European Societies of Toxicology, München, September 4 - 7

14 Blaich G and Metzler M (1988)
Modulation of 7,8-benzoflavone metabolism and enzyme activities in the male hamster liver.
Herbsttagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Hamburg, September 19 – 22

15 Blaich G and Metzler M (1988)
7,8-benzoflavone acts as a 3-methylcholanthrene-like inducer in the Syrian hamster liver model.
11th European workshop on drug metabolism, Konstanz, September 11 - 16

16 Blaich G, Tritscher AM and Metzler M (1989)
Modification of 7,8-benzoflavone metabolism in hamster liver and kidney microsomes by hepatic tumor inducing treatments.
30. Frühjahrstagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Mainz, March 14 - 17

17 Degen G, Blaich G and Metzler M (1989)
Multiple pathways for the oxidative metabolism of estrogens in Syrian hamster kidney.
5th SEK Symposium, Heidelberg, April 10 -12

18 Blaich G, Tritscher AM and Metzler M (1989)
Modification of 7,8-benzoflavone metabolism in hamster hepatic but not renal microsomes by liver tumor inducing agents.
International Conference on Environmental Mutagens, Cleveland/USA, July 10 - 15

19 Krell H, Feger R, Blaich G and Metzler M (1989)
Diethylstilbestrol (DES)-induced changes in bile secretion and DES kinetics in isolated perfused hamster liver.
1. Wintertagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Hannover, November 22 – 24

20 Blaich G, Wilke U and Metzler M (1989)
Mechanisms of hepatocarcinogenesis in male Syrian golden hamsters induced by combined treatment with estrogen and 7,8-benzoflavone.
Symposium "Mechanisms of tissue specific toxicity", Würzburg, November 19 - 22

21 Krell H, Feger R, Blaich, G and Metzler M (1989)
Diethylstilbestrol-induced changes in bile secretion and DES kinetics in isolated perfused hamster liver.
Symposium "Mechanisms of tissue specific toxicity", Würzburg, November 19 - 22

22 Schuler J, Blaich G and Metzler M (1992)
Modification of 7,8-benzoflavone metabolism in hamster liver microsomes and in vitro DNA-binding by hepatic tumor inducing treatments.
SOT-Meeting, Seattle/USA, February 23 - 27

23 Schuler J, Blaich G and Metzler M (1993)
Metabolism of α-naphthoflavone in hamster liver microsomes in vitro: effect of inducers and identification of new hydroxylation products.
5th North American ISSX Meeting, Tuscin, Arizona/USA, October 17 - 21

24 Blaich G, Marsch G and Kling M (1996)
Effect of model inducers on various P450 enzyme activities in the liver of the female dog.
37. Frühjahrstagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Mainz, March 12 - 14

25 Halm S and Blaich G (1997)
Progressive toxic tubular nephrosis induced by a cytostatic agent.
11th Annual Symposium, Gesellschaft für Toxikologie und Pathologie, Mannheim, October 10 - 11

26 Diehl K and Blaich G (1998)
Effect of different model inducers on liver UDP-GTs and serum T3, T4 and TSH levels in the male Wistar rat.
39. Frühjahrstagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Mainz, March 17 - 19

27 Blaich G and Wey A (1998)
In vivo study on xenobiotica-induced mitochondrial injury in the rat liver.
International Congress of Toxicology - ICT VIII, Paris, July 5 - 9

28 Tonkonog L, Halm S and Blaich G (2000)
Modulation of rat thyroid function by treatment with propylthiouracil (PTU).
41. Frühjahrstagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Mainz, March 21 - 23

29 Klein K-U, Blaich G and Schrenk D (2001)
Specific classification of model P450 inducers in primary hepatocyte cultures using alkoxyresorufin-O-dealkylation (AROD) and testosterone metabolism.
42. Frühjahrstagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Mainz, March 13 - 15

30 Tonkonog L, Halm S and Blaich G (2001)
Effect of a dopamine D3-receptor antagonist treatment on 5'-deiodinase activity in rat liver and kidney.
42. Frühjahrstagung der Deutschen Gesellschaft für Pharmakologie und Toxikologie, Mainz, March 13 - 15

5. References

[1] Oliver, M. F., Lancet I, 323 (1961) — [2] Levy, R. I., Morganroth, J., Rifkind, B. M., N. Engl. J. Med. 290, 1295 (1971) — [3] Galimberti, P., Defranceschi, A., Gazz. Chim. Ital. 77, 431 (1947) — [4] Merck GmbH Patent 2 130 175 of 13-3-72 — [5] Martin, R., Doctor's Thesis Abstract, University microfilm-Paris (1964) — [6] de Cointet, P., Loppinet, V., Sornay, R., Moriniere, J. L., Boucherle, A., Chim. Thér. 8, 574 (1973) — [7] Watson, D., Clin. Chim. Acta 5, 637—43 (1960) — [8] Chabrol, E., Charonnant, R., Presse Med. 45, 1713—1714 (1937)

For the authors: Dr. E. Wülfert, Centre de Recherches, Laboratoires Fournier-Dijon, 42, rue de Longvic, F-21300 Chenove (France)

ExhibitB

From Centre de Recherches, Laboratoires Fournier-Dijon, Chenove (France)

Antilipidemic Drugs

Part 2: Experimental study of a new potent hypolipidemic drug, isopropyl-[4'-(p-chlorobenzoyl)-2-phenoxy-2-methyl]-propionate (LF 178)

By J. Gurrieri, M. Le Lous, F. J. Renson, C. Tourne, H. Voegelin, B. Majoie, and E. Wülfert

Summary: *Pharmacological investigations have been carried out on a new p-chlorobenzoyl substituted phenoxy-isobutyric acid derivate with potent antilipidemic activity, isopropyl-[4'-(p-chlorobenzoyl)-2-phenoxy-2-methyl]propionate (LF 178; procetofene; Lipanthyl®).*
The compound depressed total lipids and total cholesterol significantly in the normal rat from 15—20 mg/kg upwards. At 100 mg/kg, the drug-induced depression of total lipids was twice the effect observed with 300 mg/kg of clofibrate. Significant depression of lipid parameters was induced in the senescent rat, in the dietary hyperlipidemic and in the triton hyperlipidemic rat. In the two last models clofibrate failed to affect lipid parameters at 300 mg/kg.
The absence of pharmacological side effects indicates a high pharmacological specificity with respect to lipid metabolism. It might be concluded that LF 178 presents an antilipidemic profile different from the well known hypolipidemic drug clofibrate.

Zusammenfassung: *Hypolipidämika / 2. Teil: Experimentelle Untersuchung von Isopropyl-[4'-(p-chlorbenzoyl)-2-phenoxy-2-methyl]-propionsäureester (LF 178), einem neuen hochwirksamen Hypolipidämikum*

Es wurde über pharmakologische Untersuchungen über die Wirksamkeit eines neuen p-chlorbenzoyl-substituierten Phenoxyisobuttersäure-Derivat mit lipidsenkender Wirkung berichtet, Isopropyl-[4'-(p-chlorbenzoyl)-2-phenoxy-2-methyl]-propionat (LF 178; Procetofen; Lipanthyl®).
In einer Dosis ab 15—20 mg/kg Körpergewicht senkt die Substanz die Serum-Gesamtlipide und das Gesamtcholesterin bei der normalen Ratte signifikant.
Mit 100 mg/kg LF 178 erzielt man eine Senkung der Gesamtlipide, die das Zweifache der nach Verabreichung von 300 mg Clofibrat erreichten Senkung darstellt.
Bei der alten Ratte, bei der mit Fettdiät oder mit Triton behandelten Ratte senkt LF 178 den erhöhten Blutlipidspiegel deutlich ab. Bei den zwei letzten Modellen blieb Clofibrat in einer Dosis von 300 mg/kg ohne Wirkung.
Das Fehlen pharmakologischer Nebeneffekte wird auf eine hohe pharmakologische Spezifität für den Lipidstoffwechsel zurückgeführt.
Die Ergebnisse erlauben den Schluß, daß LF 178 sich durch sein hypolipidämisches Profil von dem bekannten Hypolipidämikum Clofibrat unterscheidet.

1. Introduction

It is now widely accepted that there is a causal relationship between elevated plasma lipid levels and the development of atherosclerotic disease, which in turn leads to an increased coronary heart disease risk. Thus, it is considered therapeutically desirable to lower lipid levels within the normal range by drugs.

Clofibrate is probably the most widely used hypolipidemic drug in the western world. Its acceptance was based upon its ability to reduce levels of both cholesterol and triglycerides and upon its low toxicity. However, more than a decade of clinical experience clearly indicates that the drug has only a limited value in patients with increased light-density lipoproteins (LDL) [1].

The hypocholesterolemic activity demonstrated in animals has not been confirmed quantitatively in recent long-term clinical studies [2]. This clearly demonstrates the difficulty of selecting drugs in the field of major metabolic disorder solely on the basis of non-pathological animal model studies. However, for the purposes of developing structural requirements for maximal antilipidemic activity, a preliminary screening program of alkyl- and aryl-carbonyl-α-phenoxy-propionic acids was undertaken [3]. This project uncovered a new highly potent hypolipidemic drug, isopropyl-[4'-(p-chlorobenzoyl)-2-phenoxy]-propionate (LF 178; procetofene; Lipanthyl®)*). The present work describes the experimental studies carried out on this compound.

*) Manufacturer: Laboratoires Fournier Dijon, Chenove (France).

2. Antilipidemic activity

2.1. Methods

Fasting (water ad libitum) male Swiss rats were administered the drug to be tested in an Arabic rubber suspension (3%) by oral intubation. Control animals received the vehicle only. Blood samples were withdrawn from the retro-orbital sinus into dry tubes, centrifuged and the serum evaluated for lipid parameters. Total cholesterol was dosed colorimetrically according to Watson [4], total serum lipids by the sulfo-phosphovanillin method [5] and phospholipids evaluated after mineralization according to Briggs [6]. The Kunkel-phenol test [7] was used to determine serum lipoproteins and an alcohol-ether extraction procedure applied for quantitation of hepatic lipids. Electrophoresis was carried out on cellogel. Student and Wilcoxon tests were used for statistical evaluation.

2.2. Antilipidemic investigations in the physiologically normal rat

2.2.1. Dose-effect relationship

2.2.1.1. Method

The compounds to be tested were administered to the animals (200 g body weight) 16 and 24 h after onset of fasting (t_0, t_0 + 8 h). Blood samples were withdrawn for lipid analysis 24 h after the first intubation (t_0 + 24 h). Groups of 10 animals were used for each dose.

2.2.1.2. Results

As can be seen from Table 1, LF 178 significantly lowered total lipids when the dose administered was similar to or

Table 1: Hypolipidemic effect of LF 178 (total blood lipids) in the normal rat 24 h after oral intubation.

	Drug treated groups (lipids g/l)			Control groups (lipids g/l)			
Dose (mg/kg)	t_0	t_0 + 24 h	Δ_1	t_0	t_0 + 24 h	Δ_2	Δ_1—Δ_2
5	3.75 sm = 0.164	3.12 sm = 0.123 $p < 0.01$	—0.63	3.60 sm = 0.121	3.24 sm = 0.113 $p < 0.05$	—0.36	—0.27
10	3.58 sm = 0.114	3.17 sm = 0.117 $p < 0.025$	—0.41	3.60 sm = 0.121	3.24 sm = 0.113 $p < 0.05$	—0.36	—0.05
15	3.60 sm = 0.121	3.15 sm = 0.137 $p \simeq 0.025$	—0.45	3.60 sm = 0.121	3.24 sm = 0.113 $p < 0.05$	—0.36	—0.09
20	3.90 sm = 0.143	2.97 sm = 0.114 $p < 0.001$	—0.93	3.60 sm = 0.121	3.24 sm = 0.113 $p < 0.05$	—0.36	—0.57
50	3.41 sm = 0.133	2.88 sm = 0.155 $p < 0.02$	—0.53	4.35 sm = 0.224	4.57 sm = 0.218 NS	+0.22	—0.75
100	4.82 sm = 0.160	3.38 sm = 0.165 $p < 0.001$	—1.44	4.13 sm = 0.213	3.70 sm = 0.204 NS	—0.43	—1.01
300	3.94 sm = 0.143	3.08 sm = 0.0118 $p < 0.001$	—0.86	4.35 sm = 0.224	4.57 sm = 0.218 NS	+0.22	—1.08
Clof. 300	3.61 sm = 0.076	2.94 sm = 0.066 $p < 0.001$	—0.67	3.50 sm = 0.063	3.30 sm = 0.069 $p < 0.05$	—0.20	—0.47

Table 2: Hypocholesterolemic effect of LF 178 in the normal rat 24 h after oral intubation.

	Drug treated groups (cholesterol g/l)			Control groups (cholesterol g/l)			
Dose (mg/kg)	t_0	t_0 + 24 h	Δ_1	t_0	t_0 + 24 h	Δ_2	Δ_1—Δ_2
5	0.77 sm = 0.023	0.68 sm = 0.029 $p < 0.05$	—0.09	0.76 sm = 0.029	0.82 sm = 0.015 NS	+0.06	—0.15
10	0.81 sm = 0.0121	0.73 sm = 0.015 $p < 0.05$	—0.08	0.76 sm = 0.029	0.82 sm = 0.015 NS	+0.06	—0.14
15	0.86 sm = 0.028	0.67 sm = 0.033 $p < 0.001$	—0.19	0.76 sm = 0.029	0.82 sm = 0.015 NS	+0.06	—0.25
20	0.87 sm = 0.035	0.66 sm = 0.037 $p < 0.001$	—0.21	0.76 sm = 0.029	0.82 sm = 0.015 NS	+0.06	—0.27
50	0.77 sm = 0.021	0.61 sm = 0.026 $p < 0.001$	—0.16	0.81 sm = 0.053	0.87 sm = 0.059 NS	+0.06	—0.22
100	0.83 sm = 0.036	0.61 sm = 0.038 $p < 0.001$	—0.22	0.79 sm = 0.50	0.81 sm = 0.048 NS	+0.02	—0.24
300	0.83 sm = 0.023	0.58 sm = 0.017 $p < 0.001$	—0.25	0.81 sm = 0.053	0.87 sm = 0.059 NS	+0.06	—0.31
Clof. 300	0.93 sm = 0.018	0.56 sm = 0.016 $p < 0.001$	—0.37	0.87 sm = 0.016	0.87 sm = 0.020 NS	0.00	—0.37

exceeding 20
off at approxi
esterol was h
range (Table
mg/kg was sin

2.2.2. Kinetic
2.2.2.1. Meth
100 mg/kg of
the animals (2
(water ad libit
72 h later, an
examined for
once in order
blood volume,

2.2.2.2. Resul
The results o
were maxim
drug and ret
cholesterolem
almost 48 h
of the drug.
40%.

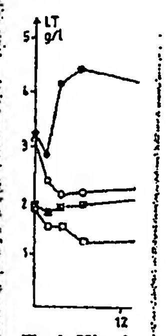

Fig. 1: Kineti
rat (100 mg/k
o——o LF 1

2.3. Antilipi
hyperlipiden
2.3.1. Meth
The animals
can be seen
higher total
mals. The d
cutive days
drawn on t
individually

2.3.2. Resul
The results
Whereas 5
significantly
depressed t
Serum chol
both dose
was appare
The antilip
decrease fr
the 1st we
(Table 5).

2.4. Antili
hyperlipide
2.4.1. Met
The animal
ed a stand
S.H.D. con
sodium ch

exceeding 20 mg/kg. The effect is dose-related and levels off at approximately 100 mg/kg. Depression of blood cholesterol was highly significant in the 15—300 mg/kg dose range (Table 2) and the maximal effect obtained at 300 mg/kg was similar to that observed with clofibrate.

2.2.2. Kinetic evaluation of hypolipidemic activity

2.2.2.1. Method

100 mg/kg of the drug was administered by oral intubation to the animals (280—300 g body weight) 24 h after onset of fasting (water ad libitum). Blood was withdrawn 2, 4, 7, 24, 31, 48 and 72 h later, and the pooled serum from groups of 3 animals examined for lipids. Each group (3 rats/group) was only used once in order to avoid interfering factors (stress, diminished blood volume, etc...).

2.2.2.2. Results

The results obtained are summarized in Fig. 1. Total lipids were maximally depressed 7 h after administration of the drug and returned to control levels within 48 h. The hypocholesterolemic effect was more sustained and lasted for almost 48 h with a maximum 24 h after oral intubation of the drug. Maximal depression of cholesterol exceeded 40%.

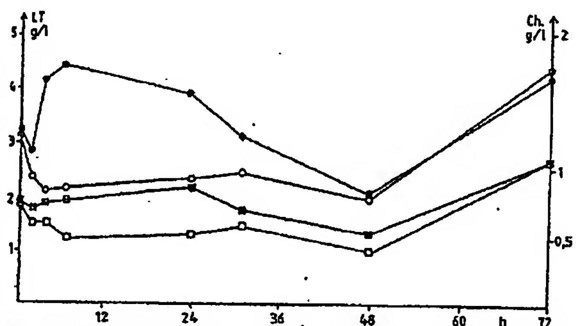

Fig. 1: Kinetic evaluation of the hypolipidemic effect of LF 178 in the rat (100 mg/kg p.o.). Left ordinate: total lipids ●——● controls, ○——○ LF 178; right ordinate ■——■ controls, □——□ LF 178.

2.3. Antilipidemic investigations in the physiologically hyperlipidemic rat (senescent rats)

2.3.1. Method

The animals used in this study were 24 months old rats. As can be seen from Table 3 and 4, these animals had significantly higher total lipid and cholesterol values than had younger animals. The drug was administered (10 rats/dose) for 4 consecutive days a week for a period of 3 weeks. Blood was withdrawn on the fifth day of each week and the sera analysed individually for total lipids, cholesterol and lipoproteins.

2.3.2. Results

The results obtained are summarized in Tables 3, 4 and 5. Whereas 50 mg/kg per day failed to depress total lipids significantly before the third week, twice that dose already depressed total lipids after 4 days of treatment.

Serum cholesterol was, however, significantly depressed at both dose levels after 4 days of treatment and this effect was apparently dose dependent.

The antilipidemic effect was also reflected in the transient decrease in lipoproteins (optical density evaluation) after the 1st week of treatment at the 100 mg/kg dose level (Table 5).

2.4. Antilipidemic investigations in the dietary hyperlipidemic rat

2.4.1. Method

The animals (14 per group, 170 g body weight) were administered a standard hyperlipidemic diet (S.H.D.) for 4 weeks. 100 g S.H.D. contained: 38 g butter, 20 g casein, 2 g cholesterol, 0.8 g sodium cholate, 7 g of minerals, 1 g vitamins, 0.2 g choline and excipient dextrose. The first two weeks the animals were fed the diet only, the last two weeks, the regimen was given concomitantly with the drug to be studied. Food consumption was carried out every day and weight control once a week. The fasting animals were decapitated 8 h after the last administration, total serum lipids, serum cholesterol, serum turbidity and transaminases evaluated and gross pathology, organ weight and histopathological studies (liver, kidney, spleen, lungs, heart, aortic cross, thoracic aorta, abdominal aorta) carried out.

2.4.2. Results

From Table 6 it can be seen that only LF 178 at 50 mg/kg significantly depressed total lipids and serum cholesterol. The concomitant reduction in serum turbidity reflected the antilipidemic effect of the drug. A six times higher dose of clofibrate (300 mg/kg) did not affect any of the lipid parameters studied.

Table 7 shows that the antilipidemic effect did not have an anorexic origin, food consumption being unchanged by the drug. A weak but significant elevation of SGPT was observed with both drugs. Organ examination did not reveal any drug induced anomality but the typical gain in liver weight well known with this class of drugs.

Table 3: Antilipidemic effect (total blood lipids g/l) of LF 178 in the senescent rat.

	t_0	1st week	2nd week	3rd week
Control	5.9 sm = 0.92 *)	5.8 sm = 0.97 NS*)	6.0 sm = 1.04 NS*)	6.1 sm = 0.85 NS*)
LF 178 50 mg/kg	4.9 sm = 0.62 NS*)	4.2 sm = 0.41 NS	4.6 sm = 0.51 NS	3.7 sm = 0.36 Δ = 1.2 $p < 0.05$
LF 178 100 mg/kg	4.5 sm = 0.36 NS*)	3.3 sm = 0.16 Δ = 1.2 $p < 0.01$	4.0 sm = 0.46 NS	3.8 sm = 0.41 NS

*) Compared to control values.

Table 4: Hypocholesterolemic effect of LF 178 in the senescent rat (cholesterol g/l).

	t_0	1st week	2nd week	3rd week
Control	1.45 sm = 0.239 *)	1.45 sm = 0.233 NS*)	1.37 sm = 0.211 NS*)	1.35 sm = 0.202 NS*)
LF 178 50 mg/kg	1.38 sm = 0.175 NS*)	1.06 sm = 0.122 Δ = 0.32 $p < 0.05$	1.12 sm = 0.154 Δ = 0.26 $p < 0.05$	0.88 sm = 0.134 Δ = 0.50 $p < 0.02$
LF 178 100 mg/kg	1.35 sm = 0.201 NS*)	0.84 sm = 0.052 Δ = 0.51 $p < 0.02$	0.97 sm = 0.099 NS	1.06 sm = 0.112 NS

*) Compared to control values.

Table 5: Effect of LF 178 on lipoprotein levels in the senescent rat. Results are expressed in optical density units.

	t_0	1st week	2nd week	3rd week
Control	0.18 sm = 0.054	0.18 sm = 0.053 NS*)	0.19 sm = 0.037 NS*)	0.17 sm = 0.042 NS*)
LF 178 50 mg/kg	0.13 sm = 0.032 NS*)	0.11 sm = 0.017 NS	0.125 sm = 0.025 NS	0.13 sm = 0.030 NS
LF 178 100 mg/kg	0.13 sm = 0.014 NS*)	0.08 sm = 0.005 Δ = 0.05 $p < 0.01$	0.10 sm = 0.017 NS	0.13 sm = 0.017 NS

*) Compared to control group

Table [illegible]: Hypolipidemic effect of LF 178 in the dietary hyperlipidemic rat after 14 days of treatment.

	Total lipids (g/l)	Total cholesterol (g/l)	Turbidity	SGOT (U/l)	SGPT (U/l)
Control group	11.2 sm = 0.84	4.34 sm = 0.42	1.5	214 sm = 14.3	59 sm = 3.4
Clofibrate 300 mg/kg	10.3 sm = 0.73 NS	3.76 sm = 0.34 NS	1	236 sm = 26.8 NS	91 sm = 4.8 p < 0.001
LF 178 25 mg/kg	10.0 sm = 1.00 NS	3.53 sm = 0.52 NS	1.5	253 sm = 27.4 NS	71 sm = 5.1 NS
LF 178 50 mg/kg	8.5 sm = 0.60 p < 0.02	3.16 sm = 0.26 p < 0.05	0.5	252 sm = 25.9 NS	111 sm = 17.5 p < 0.01

Table 7: Effect of drug treatment on daily food consumption in g/group.

	Control group	Clofibrate 300 mg/kg	LF 178 25 mg/kg	LF 178 50 mg/kg
Food consumption (diet only)	184 sm = 5.7	191 sm = 3.8 NS	191 sm = 5.2 NS	189 sm = 4.5 NS
Food consumption (diet and drug treatment)	178 sm = 6.1	165 sm = 5.4 NS	175 sm = 4.1 NS	169 sm = 5.8 NS
J + 14 Final	299 sm = 9.6	299 sm = 8.1 NS	298 sm = 5.1 NS	292 sm = 4.3 NS

2.5. Antilipidemic investigations in triton-treated hyperlipidemic rats

2.5.1. Method

The technique was that used by Garattini [8]. Fasting rats (200 g body weight) were made hyperlipidemic 6 h after onset of fasting by an i.v. injection of a physiological saline solution containing 4% of triton W.R. 1339. The drugs to be studied were given by oral intubation immediately after and lipid parameters evaluated 18 h later. Clofibrate was inactive in this test. Nicotinic acid was therefore used as reference compound. Groups of 10 animals were used for each dose.

2.5.2. Results

It can be seen from Table 8 that LF 178 had a highly significant antilipidemic effect at 100 and 300 mg/kg. Whereas total lipids and lipoproteins were depressed at both doses, cholesterol values were only reduced significantly at the highest dose (300 mg/kg). Although not statistically significant, the antilipidemic effect was apparent at the 30 mg/kg dose level. The reference compound (nicotinic acid) was less active than LF 178 (100 mg/kg).

Table 8: Hypolipidemic effect of LF 178 in triton treated rats. Clofibrate was inactive in this test.

	Total lipids (g/l)	Lipo-proteins (Kunkel phenol)	Total cholesterol (g/l)
Control group	9.1 sm = 0.63	0.70 sm = 0.159	1.20 sm = 0.074
Nicotinic acid 500 mg/kg	6.6 sm = 0.39 p < 0.01	0.31 sm = 0.021 p < 0.01	0.96 sm = 0.049 p < 0.02
LF 178 30 mg/kg	7.8 sm = 0.81 NS	0.43 sm = 0.067 NS	1.22 sm = 0.081 NS
LF 178 100 mg/kg	5.4 sm = 0.41 p < 0.01	0.22 sm = 0.042 p < 0.01	1.06 sm = 0.043 NS
LF 178 300 mg/kg	5.2 sm = 0.25 p < 0.01	0.19 sm = 0.022 p < 0.01	0.94 sm = 0.040 p < 0.02

3. Investigation on side effects

3.1. Methods

Anti-inflammatory activities were assessed by the inhibition of edema formation were carried out on male rats (10 rats per group, 150 g body weight) in response to a subplantar injection of carrageenin (1%, 0.1 ml). The experimental procedure followed was that of Winter et al. [9].
Gastric tolerance of LF 178 and LF 153 (the corresponding phenoxy carboxylic acid) was assessed in the male rat (10 rats per group, 125—135 g body weight). The drugs were administered 6 days a week for 2 weeks by oral intubation. At the end of the study, the animals were sacrificed and their stomach examined for gastric ulcer (microscopic examination; for rating, see Table 9).
Investigation on cardiovascular parameters was carried out in the dog.
The animals were kept under pentobarbital-Na anesthesia throughout the experiments. The drug (20 mg/kg) was administered to the animals via the saphenous vein after solubilization in dimethylsulfoxide (DMSO). The corresponding phenoxycarboxylic acid (LF 153) was administered at 5, 10, 20 mg/kg as the sodium salt in an equal volume of physiological saline solution. Femoral arterial blood pressure, D_2-derivated ECG, heart frequency and the amplitude of the QRS ventricular complex were recorded. The response of treated animals was compared with that obtained with the vehicle only. Studies on basic metabolic rate were carried out on male rats (10 rats per group, 220 g body weight) in a typical Bargeton chamber (measure of O_2-consumption) after 10 days of treatment with the drug (100 mg/kg per day).
CNS effects were evaluated in the actimeter and in the evasion-test (exploratory activity of the mouse) according to Boissier and Simon [10]. Anticonvulsive activity was evaluated as the percent inhibition of pentetrazol induced convulsions. Animal behaviour was controlled currently in acute and long-term toxicity studies.
Peripheral autonomic effects were assessed as usual (pupillary diameter, salivation, defecation, piloerection) and spasmolytic activity studied on an isolated ileum guinea pig preparation.

3.2. Results

No anti-inflammatory effect could be observed with LF 178 in the dose range studied (50—400 mg/kg). The corresponding phenoxy-isobutyric acid (LF 153) (major circulating metabolite in man) had a significant effect at doses exceeding 200 mg/kg (see Fig. 2) (BD_{50} = 250 ± 80 mg/kg).
The results obtained in the evaluation of gastric tolerance are summarized in Table 9. Whereas LF 178 is almost

SGPT (U/l)
59 = 3.4
91 n = 4.8 p <0.001
71 = 5.1 NS
111 = 17.5 p < 0.01

F 178 mg/kg
189 ı = 4.5 NS
169 ı = 5.8 NS
292 ı = 4.3 NS

inhibition of (10 rats per ntar injection rocedure fol-

:orresponding ı rat (10 rats were admini- ation. At the :heir stomach n; for rating,

arried out in

a anesthesia kg) was ad- ıfter solubili- onding phen- i, 10, 20 mg/ logical saline ivated ECG, ventricular animals was '. Studies on (10 rats per on chamber atment with

the evasion- to Boissier ıated as the ons. Animal ıg-term toxi-

ıl (pupillary spasmolytic reparation.

ith LF 178 :orrespond- circulating ses exceed- ıg/kg). : tolerance is almost

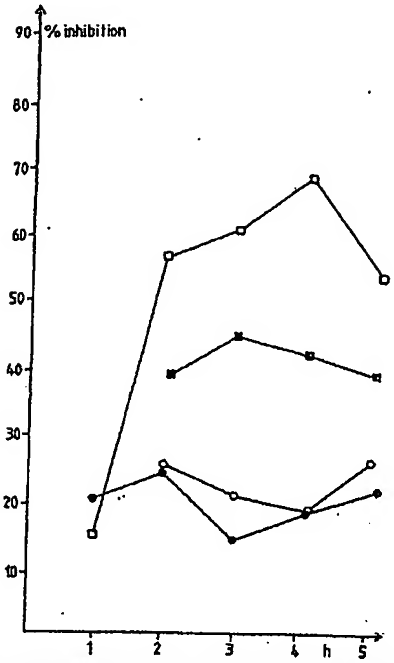

Fig. 2: Anti-inflammatory effect of LF 153 in the carrageenin edema test (rat). ●——● 50 mg/kg; ○——○ 100 mg/kg; ■——■ 200 mg/kg; □——□ 400 mg/kg.

Table 9: Gastric tolerance. ++++ profound and extended ulceration; +++ isolated islets of profound ulceration; ++ extended but superficial ulceration; + isolated superficial ulcerations; 0 no ulcer observed.

Animal code	LF 178 (300 mg/kg)				LF 153 (300 mg/kg)			
	+		+++	++++	+	++	+++	++++
T	0	0	0	0	4	0	0	0
Q	3				20	0	0	0
TQ	0	0	0	0	8	2	1	0
D	0	0	0	0	1	0	0	0
B	1	0	0	0	0	3	0	0
TV	0	0	0	0	9	0	1	2
QV	0	0	0	0	4	0	0	0
TQV	2	0	0	0	1	0	0	0
DV	0	0	0	0	9	0	2	0
BV	2	0	0	0	5	0	0	0
Total	8				60	6	4	2

devoid of any ulcerogenic effect, the acid metabolite LF 153 did induce significant lesions at 300 mg/kg. The cardiovascular investigations did not reveal any effect either of LF 178 or of LF 153 (Table 10).

It should be mentioned that the plasma peak concentration observed in these experiments (100—400 μg/ml of LF 153) is at least 10—40 times higher than the plasma steady state level of the acid metabolite observed in man after several months of treatment [11].

Table 11 clearly shows that the drug did not interfere with oxygen consumption under the conditions used.

Table 10: Cardiohemodynamic effects of LF 153 (sodium salt).

Dose (mg/kg) i.v.	Dogs	Heart rate	Systolic arterial pressure	Diastolic arterial pressure	$P_s - P_d$	Mean aortic pressure	Left intraventricular pressure	$\frac{dp/dt}{p}$	Aortic flow	Femoral flow	Systemic vascular resistance	Stroke volume (S.V.)	Stroke time (S.T.)	Isometric contraction time	Tension time index (T.T.I.)	Stroke work	Cardiac work
5	Dog 1 ♂ 13 kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.
	Dog 2 ♀ 11.2 kg	—10%	0	≃0	≃0	0	0	0	0	≃0	0	+10%	0	0	0	+12,5%	0
	Dog 3 ♂ 13 kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	.0	0
	Dog 4 ♂ 17 kg	0	0	0	0	0	0	0	—8%	0	+8%	—8%	0	0	0	—8%	—8%
	Dog 5 ♂ 13.6 kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Dog 1 ♂ 13 kg	+10%	0	0	0	0	0	0	0	0	0	—10%	0	0	0	—10%	0
	Dog 2 ♀ 11.2 kg	—12%	0	0	0	0	≃0	≃0	0	0	0	+14%	+14%	0	0	+14%	0
	Dog 3 ♂ 13 kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dog 4 ♂ 17 kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dog 5 ♂ 13.6 kg	0	0	0	0	0	0	/	—12%	0	+12%	—12%	0	0	0	0	—12%
20	Dog 1 ♂ 13 kg	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Dog 2 ♀ 11.2 kg	—12%	0	0	0	0	0	0	—11%	0	+11%	0	0	0	—12%	0	—11%
	Dog 3 ♂ 13 kg	0	0	0	0	0	0	0	—12%	0	+12%	—12%	0	0	0	—12%	—12%
	Dog 4 ♂ 17 kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dog 5 ♂ 13.6 kg	+8%	0	0	0	0	0	0	0	0	0	—8%	0	0	0	0	0

Table 11: Effect of LF 178 on basic metabolic rate. Consumption of O_2 (ml)/5 min. sm = standard deviation from the average.

	Control group	Treated group
A) Before treatment	34.95 ml sm = 1.36	34.34 ml sm = 0.94
B) After 10 days of treatment	36.31 ml sm = 0.75	36.43 ml sm = 1.25
B — A	1.36 ml sm = 1.46	2.09 = ml sm = 1.25

No effect on animal behaviour (rat and mouse) or any sypmtoms related to an interaction with central or peripheral nervous system mechanisms could be recorded throughout the toxicity studies even with oral doses exceeding 3200 mg/kg. The compounds were devoid of effect in the specific test systems used. LF 153 (10 and 20 mg/kg i.v.) did neither affect the norepinephrine induced pressor effect in the anesthetized dog, nor relax the guinea pig ileum preparation in vitro (10^{-6}—10^{-2} M).

4. Discussion

The results obtained throughout the antilipidemic studies clearly demonstrate that LF 178 is a potentially active hypolipidemic agent. While being more active than clofibrate on total lipids in the normal rat, the anticholesterolemic potency was only similar to or slightly less than the reference compound (Table 1 and 2).

It is reasonable to hypothesize that in man a true hypocholesterolemic agent would affect LDL more specifically than VLDL. This should be borne in mind when screening for antilipidemic drugs in rats where LDL only play a minor part in lipid metabolism. The senescent rat was supposed to be a more relevant experimental approach to human hyperlipidemia. Plasma lipid levels are significantly higher in these animals. Although clofibrate was not studied in this model, LF 178 appeared to have potential hypocholesterolemic activity at 50 mg/kg (Table 4). At 100 mg/kg, however, the compound only brought about a transient decrease in cholesterol after 4 days of treatment. The depression of total lipids followed a similar pattern (Table 3). Some feed-back regulatory mechanims might well be operating at the level of cholesterol metabolism when drug induced depressions of circulating cholesterol levels are fast and well pronounced. A similar compensatory effect (return to physiological pretreatment levels) has been observed currently in our laboratory when treating normal rats with antilipidemic agents for several weeks. This clearly focuses on the relevance of normal, non-pathological animals as a model when searching for new drugs in the field of human metabolic disease. A significant difference in potency between clofibrate and LF 178 was apparent in experiments carried out on the dietary hyperlipidemic rat.

While clofibrate was inactive in this test, LF 178 depressed both total lipids and total cholesterol at the 50 mg/kg dose level (Table 6). The increase in transaminase (SGPT) was observed with both drugs and therefore not related to the antilipidemic effect. Long-term toxicity studies (6 months in the rat) have shown that the transaminase levels (SGPT) return to normal after 2 months of treatment. A glance at Table 7 clearly indicates that the hypolipidemic effect was specific and not related to any change in food consumption. Gross pathology and histo-pathological studies did not reveal any drug induced anomality. Significant liver enlargement was, however, recorded. This is a well documented side effect of hypolipidemic aryl-phenoxy-butyric acids in rodents [12].

The hepatomegaly which was rapidly reversible after withdrawal of the drug is probably species dependent because no such effect could be observed in the dog, even after 2 years of treatment (toxicity studies). A further difference in pharmacological specificity between clofibrate and LF 178 showed up in the triton treated rat. Whereas clofibrate was devoid of activity under the conditions used, LF 178 induced a significant decrease of all parameters studied. Nicotinic acid used as reference compound, was less active than LF 178. Although the value of this model in selecting new drugs is debatable, the experimental data indicate that LF 178 might behave differently from clofibrate in human disease with regard to potency and therapeutic specificity.

6. References

[1] Levy, R. I., Morganroth, J., Rifkind, B. M., N. Engl. J. Med. 23, 297, 1295 (1974) — [2] J. Amer. Med. Ass. 231, 360 (1975) — [3] Sornay R., Gurrieri, J., Tourne, C., Renson, F. J., Majoie, B., Wülfert, E., Arzneim.-Forsch. (Drug Res.) 26, 885 (1976) — [4] Watson, D., Clin. Chim. Acta 5, 637 (1960) — [5] Chabrol, E., Charonnat, R., Presse Med. 45, 1713—1714 (1937) — [6] Briggs, A. P., Fiske, Ch., Subbarow, Y., J. Biol. Chem. 66, 375 (1926) — [7] Kunkel, H. G., Proc. Soc. Exp. Biol. Med. 66, 217—24 (1947) — [8] Garattini, S., Morpurgo, C., Paoletti, P., Paoletti, R., Arzneim.-Forsch. (Drug Res.) 9, 206 (1959) — [9] Winter, C. A., Ann. Rev. Pharmacol. 6, 157 (1966) — [10] Boissier, J. R., Simon, P., Therapie 1, 1170—1175 (1960) — [11] Tourne, C., Wülfert, E., to be published — [12] Howe, R., in: Advances in Drug Research, vol. 9, ed. N. J. Harper and A. B. Simmonds, Acad. Press., New York, 1974

For the authors: Dr. E. Wülfert, Centre de Recherches, Laboratoires Fournier-Dijon, 42, rue de Longvic, F-21300 Chenove (France)

From U.E.R. des Sciences Pharmaceutiques et Biologiques de Grenoble (France)

Antilipidemic Drugs

Part 3: On the synthesis of ^{14}C-radiolabelled isopropyl-[4'-(p-chlorobenzoyl)-2-phenoxy-2-methyl]-propionate (LF-178)

By C. Luu Duc

Summary: *Isopropyl-[4'-(p-chlorobenzoyl)-2-phenoxy-2-methyl]-propionate (LF 178; procetofene; Lipanthyl®) was labelled with ^{14}C. The carbon atom of the ketone function was chosen for the study.*

The obtained product has a total specific radioactivity of 8.79 mCi/mM. Its structure was proved by NMR.

Zusammenfassung: *Antilipidämika / 3. Teil: Über die Herstellung von ^{14}C-markiertem Isopropyl-[4'-(p-chlorbenzoyl)-2-phenoxy-2-methyl]-propionsäureester*

Isopropyl-[4'-(p-chlorbenzoyl)-2-phenoxy-2-methyl]-propionsäureester (LF 178; Procetofen; Lipanthyl®) wurde mit ^{14}C markiert. Das C-Atom der Keton-Funktion wurde als Markierungsposition gewählt.

Die spezifische Aktivität der Ausgangssubstanz betrug 8,79 mCi/mM. Ihre Struktur wurde durch NMR bestätigt.